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# *Russula changbaiensis* sp. nov. from northeast China

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Abstract —*Russula changbaiensis* (subg. *Tenellula* sect. *Rhodellinae*) from the Changbai Mountains, northeast China, is described as a new species. It is characterized by the red tinged pileus, slightly yellowing context, small basidia, short pleurocystidia, septate dermatocystidia with crystal contents, and a coniferous habitat. The phylogenetic trees based on ITS1-5.8S-ITS2 rDNA sequences fully support the establishment of the new species.

Key words —*Russulales*, *Russulaceae*, taxonomy, morphology, *Basidiomycota*

## Introduction

The worldwide genus of *Russula* Pers. (*Russulaceae*, *Russulales*) is characterized by colorful fragile pileus, amyloid warty spores, abundant sphaerocysts in a heteromerous trama, and absence of latex (Romagnesi 1967, 1985; Singer 1986; Sarnari 1998, 2005). As a group of ectomycorrhizal fungi, it includes a large number of edible and medicinal species (Li et al. 2010). The genus has been extensively investigated with a long, rich and intensive taxonomic history in Europe (Miller & Buyck 2002). Although *Russula* species have been consumed in China as edible and medicinal use for a long time, their taxonomy has been overlooked (Li & Wen 2009, Li 2013). Only 14 new species and three new varieties have been reported from China (Singer 1935; Chiu 1945; Ying 1983, 1989; Bi & Li 1986; Zang & Yuan 1999; Wen & Ying 2001; Wang et al. 2009; Li et al. 2011, 2012), and a systematic investigation of the *Russulaceae* in China has also been limited until recently (Wang & Liu 2009, 2010; Das et al. 2010; Buyck & Atri 2011; Das & Verbeken 2011; Wang et al. 2012).

The Changbai Mountain range on the borders of China, Korea, and Russia, is considered a region highly diverse in macro-fungi (Xie et al. 1986). During

a foray in the Changbai Mountains, several interesting *Russula* specimens were collected; after thorough examination, they are described here as a new species.

## Materials & methods

Fresh specimens were photographed using a Sony Cyber Shot DSC-S75 digital camera, and macroscopic characteristics of only the intact fresh fruitbodies were recorded under sunlight in the field. After being dried at 50–60 °C in a SIGG Dorrex food dehydrator, the specimens were deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS). Small samples of fresh fruit bodies were dried in silica gel for DNA extraction. Color names and codes follow Ridgway (1912). Microscopic characters were examined under a Nikon Eclipse 80i microscope according to Yang (2000), Wang et al. (2009) and Li et al. (2012). Sulfovanillin (SV) solution was used to test chemical reactions of the rehydrated specimens.

Protocols for DNA extraction, PCR, and sequencing followed those in Li et al. (2012) and references therein. Sequences of the internal transcribed spacer (ITS) region were amplified with the primer pairs ITS1/ITS4 (Gardes & Bruns 1993). PCR products were purified with the Bioteke DNA Purification Kit (Bioteke, Beijing, China). ITS regions were sequenced with the ABI 3730 DNA analyzer and ABI Bigdye 3.1 terminator cycle sequencing Kit (BGI, Beijing, China). Sequences obtained were submitted to GenBank (accession numbers are shown in Fig 3). Other sequences of representative and closely related *Russula* taxa (Eberhardt 2002; Miller & Buyck 2002; Li et al. 2011, 2012; Li 2013) were retrieved from GenBank. Assembly and editing of sequences of each region were performed with Clustal X and BioEdit (Thompson et al. 1997, Hall 1999) and were manually modulated when necessary. Some ambiguously aligned terminal sites were excluded in sequence alignment.

Based on previous phylogenetic works (Eberhardt 2002, Miller & Buyck 2002, Ryman et al. 2003, Lebel & Tonkin 2007), *Albatrellus flettii* Morse ex Pouzar and *Gloeocystidiellum aculeatum* Sheng H. Wu were chosen as outgroup taxa.

Maximum Parsimony (MP) analysis of the phylogenetic relationships among the taxa were performed in PAUP* v.4.01 (Swofford 2004). Gaps in alignment were treated as missing data, and all sites were treated unordered and unweighted. The tree bisection-reconstruction (TBR) algorithm was performed with the heuristic search option, and bootstrap analysis was conducted with 1000 replicates (Felsenstein 1985). Consistency index (CI), retention index (RI), and tree length (TL) were also calculated. Trees were displayed with Treeview 1.6.6 (Page 1996).

## Taxonomy

***Russula changbaiensis*** G.J. Li & H.A. Wen, **sp. nov.** Figs 1-2

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Differs from *Russula puellula* by wider pileocystidia with crystal contents.

Type: China, Jilin Province, Antu County, Erdaobaihe, Heping Forest Farm, 43°07′N 128°54′E, alt. 1014 m, in coniferous forest dominated by *Abies nephrolepis*, 22.VII.2010, L.D. Guo, X. Sun, G.J. Li & L.J. Xie CBS20100344 (**Holotype**, HMAS262369, GenBank KC412162); CBS20100431 (**Isotype**, HMAS262394; GenBank KC412165).

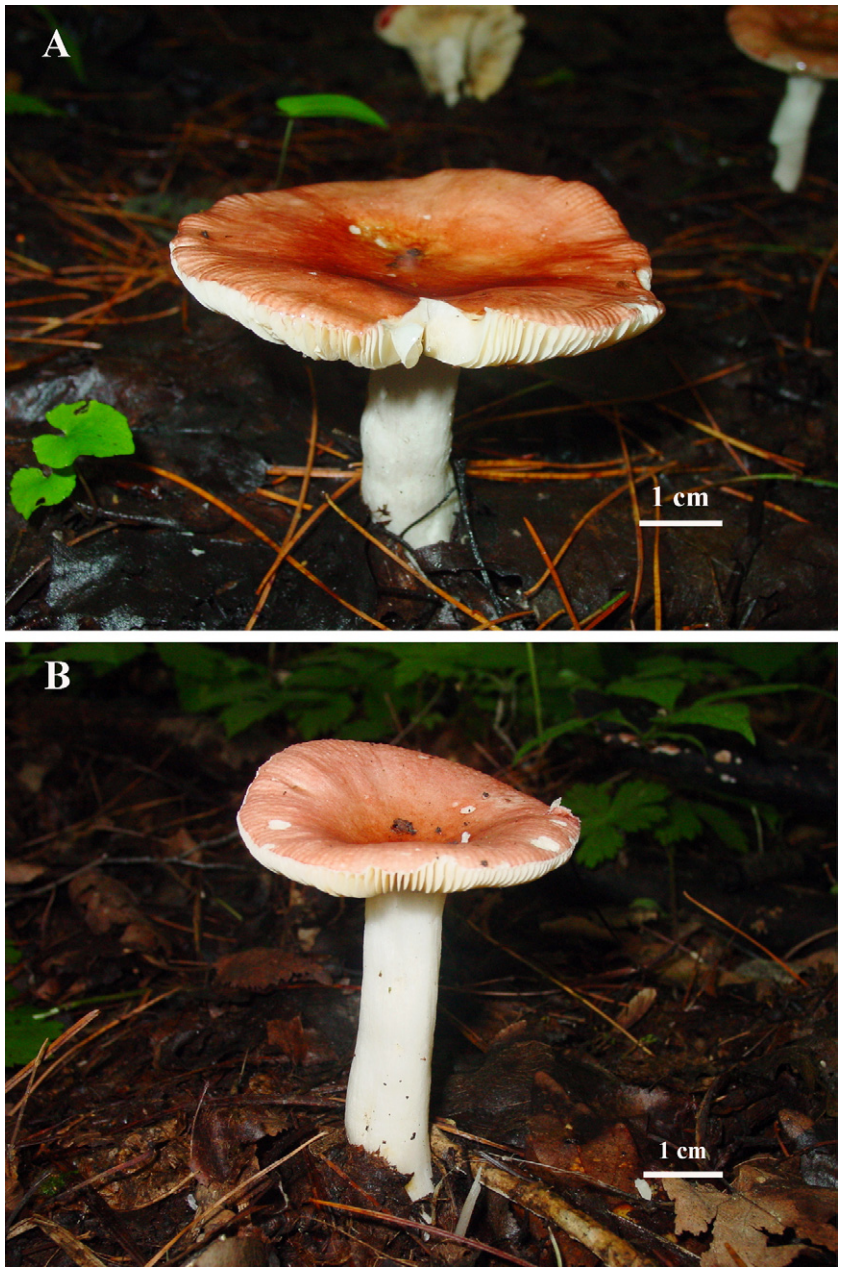


Fig. 1: *Russula changbaiensis* A. Holotype HMAS 262369; B. HMAS 262355.

Etymology: named after the mountains of the type locality.

Basidiomata small to medium sized, slender and fragile. Pileus 2–6 cm in diam., first hemispherical, then convex to applanate, sometimes slightly depressed above the stipe, or even shallow infundibuliform, Dark Vinaceous (xxvii1″) to Corinthian Red (xxvii3″) at centre rarely with small patches of Pale Yellow-Orange (iii15f), glossy, Prussian Red (xxvii5″k) to Indian Red (xxvii3″k) when dry, viscous when humid; margin incurved first, straight to slightly up-curled when mature, sometimes undulate and dehiscent, slightly striate, Pale Vinaceous (xxvii1″f) to Hydrangea Pink (xxvii1″i), Deep Corinthian Red (xxvii3″i) to Ocher Red (xxvii5″b) when dry; cutis 1/2–3/4 separable from pileus edge, sometimes with small part exfoliating. Lamellae up to 5 mm height in middle, adnate to slightly subfree, rarely forked near the stipe, slightly interveined, Cream Color (xvi19′f), brittle, Naples Yellow (x19′d) when dry; edge even, narrowing towards the pileus margin, 11–13 pieces per cm in the edge; lamellulae absent. Stipe 3–5 × 1–1.5 cm, central, smooth, subclavate to cylindrical, sometimes a little ventricose toward the base, without annulus, White (liii), partly turning Baryta Yellow (iv21f) when aged and dry, rarely with small Cinnamon (xxix15″) spots at the base, not pruinose, fragile, hollow when mature. Context 2–4 mm thick at the pileus center, White (liii), turning slightly Sulphur Yellow (v25f) when old and injured; taste mild to slightly acrid, moderately acrid in gills; no distinct odor. Spore print Pale Cream to Cream (Romagnesi iia–iib).

Basidiospores [100/5/5] (6.6–)6.9–7.9(–8.1) × (5.8–)6.0–6.6(–7.3) μm, Q = (1.03–)1.11–1.24(–1.28) (**Q** =1.19 ± 0.05), subglobose to broadly ellipsoid, verrucose; ornamentation amyloid, composed of warts mostly fused into fine lines, cristulate to subreticulate, with some isolated verrucae, less than 1.0 μm high; plage distinctly amyloid. Basidia 26–34 × 8–13 μm, clavate, inflated towards upper half, 4–spored, projecting 5–10 μm beyond hymenium, hyaline, smooth; sterigmata 2–4 μm, pointed, straight to slightly curved. Pleurocystidia few, 43–56 × 6–10 μm, originating in subhymenium, projecting 25–30 μm beyond hymenium, thin–walled, clavate to subfusiform, with refractive contents grayish in SV in middle and lower parts; apices obtuse, often with a moniliform to papillate appendage. Cheilocystidia not observed; lamellar edge sterile. Pileipellis two layered. Epicutis a trichoderm 150–250 μm thick, composed of 3–4 μm thick, slender hyaline hyphae, occasionally with intracellular pigmentation particles. Pileocystidia numerous, 70–130 × 6–8 μm, originating in subcutis, clavate, slender, septate, with crystal contents ash black in SV; apices obtuse. Subcutis a cutis composed of recumbent, hyaline, 2.5–4 μm diam hyphae. Trama composed of connective hyphae 2–5 μm diam and hyaline, globose to subglobose, 25–40 μm diam sphaerocystsis. Stipitipellis a cutis not well developed, composed of thin–walled, septate,

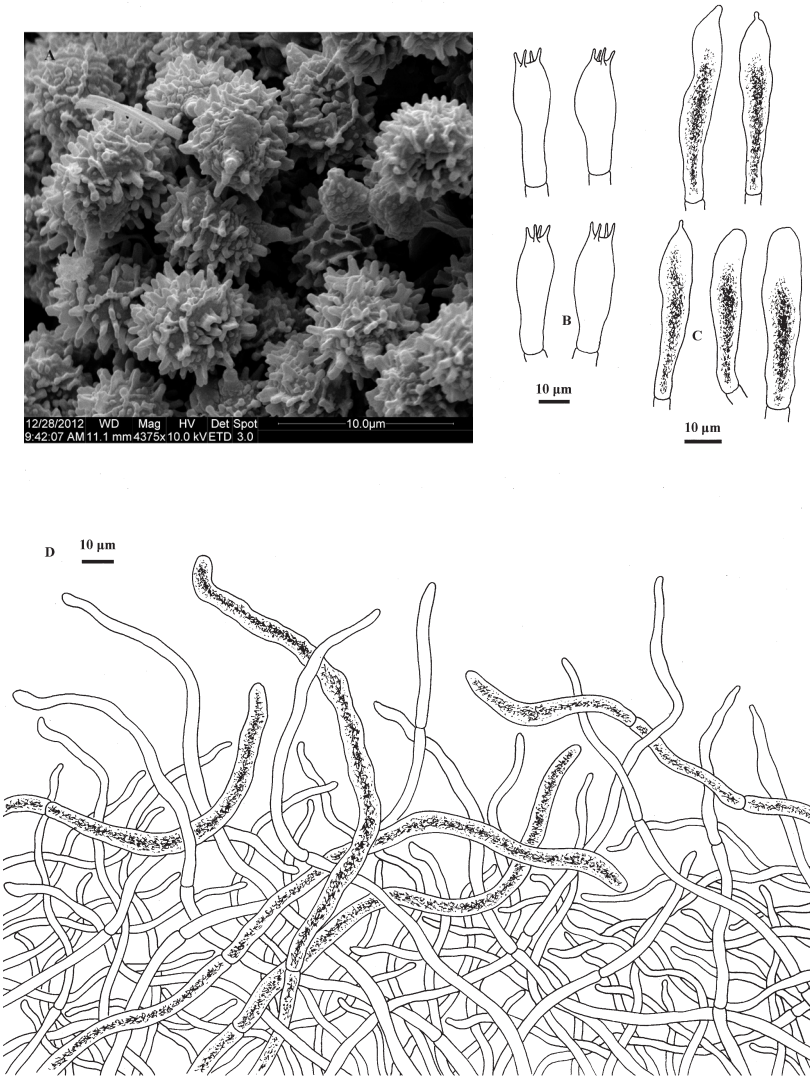


Fig. 2 *Russula changbaiensis* HMAS 262369 (holotype).
A. Basidiospores (SEM); B. Basidia; C. Pleurocystidia; D. Pileipellis.

cylindrical, hyaline hyphae 3–6 µm wide; caulocystidia absent. Clamp connections absent in all tissues.

Ecology & distribution: Solitary in conifer and conifer-broadleaf forest (often has a preference for *Abies* spp.). Distribution: China (Jilin). Season: July.

Other specimens examined: **China**, **Jilin Province**, Antu County, Hepingyingzi, 43°07′N 128°54′E, alt. 882 m, in mixed coniferous-broadleaf forest, 21.VII.2010, L.D. Guo, X. Sun, G.J. Li & L.J. Xie CBS20100044 (HMAS262381, GenBank KC412164); CBS20100514 (HMAS262376, GenBank KC412163); Changbai Mountains Forest Ecological System Research Center, 43°23′′N 128°05′E, alt. 811 m, in mixed coniferous-broadleaf forest, 25.VII.2010, X. Sun & G.J. Li CBS20100299(HMAS262355, GenBank KC412161).

Comments: The combination of slender basidiomata, red tinged pileus with slightly striate margin, separable pileipellis, small basidia, few short pleurocystidia, and pluriseptate dermatocystidia placed this new species in sect. *Rhodellinae* Romagn. in *Russula* subg. *Tenellula* Romagn. *Russula changbaiensis* is characterized by its slightly yellowing context, cream spore print, dermatocystidia with crystal contents, and coniferous habitat. The nine other species with a reddish tinged pileus in sect. *Rhodellinae* are *R. arpalices* Sarnari, *R. conviviales* Sarnari, *R. font-queri* Singer, *R. impolita* (Romagn.) Bon, *R. melzeri* Zvára, *R. pseudoimpolita* Sarnari, *R. puellula* Ebbesen et al., *R. rhodella* E.-J. Gilbert, and *R. zonatula* Ebbesen & Jul. Schäff. (Romagnesi 1967, 1985; Sarnari 2005). All are differentiated from *R. changbaiensis* as follows: *Russula arpalices* has a strongly yellowing context and distinctive *Pelargonium*-like odor; *R. conviviales* has an ocher spore print, partly subulate epicutis terminal cells, and wider pileocystidia (8–12 µm, Sarnari 2005) with golden yellow contents; *R. font-queri* has obviously yellowish-orange tinged pileus and lamellae, pale red tinged stipe, yellow spore print, context with a taste of old fruit, longer basidia (28–50 × 8.5–12.5 µm, Romagnesi 1967), and pleurocystidia (40–85 × 7–12 µm, Romagnesi 1967); *R. impolita* has an opaque pileus cutis, fruity odor, spores with isolated warts, and finely encrusted dermatocystidia; *R. melzeri* has an epicutis composed of large and short hyphal cells growing from a subcutis made up of sphaerocyst-like cells; *R. pseudoimpolita* has a velutinous pileus, barely changing context, ampullaceous pileipellis hyphal terminals, and association with *Quercus; R. puellula* has larger hyaline basidia, slender dermatocystidia (3–6 µm, Sarnari 2005), and an association with beech and oak deciduous forest; *R. rhodella* has an ocher to pale yellow spore print, and isolated spore ornamentation; *R. zonatula* is recognized for its obviously discolored pileus, yellowish spore print, isolated spore ornamentation, pleurocystidia over 90 µm long, and an association with beech forest (Romagnesi 1967, 1985; Sarnari 2005).

*Russula* taxa with reddish tinged pilei originally described from China are *R. chichuensis* W.F. Chiu, *R. griseocarnosa* X.H. Wang et al., *R. handelii* Singer, *R. jilinensis* G.J. Li & H.A. Wen, *R. minutula* var. *minor* Z.S. Bi, *R. taliensis* W.F. Chiu, *R. pseudovesca* J.Z. Ying, *R. punicea* W.F. Chiu, and *R. zhejiangensis* G.J. Li & H.A. Wen. It is easy to discriminate between these species and *R. changbaiensis: R. chichuensis* has a white spore print, large pleurocystidia ≤130

μm long, and no pileocystidia; *R. griseocarnosa* differs in the larger pileus (5–12 cm diam.), slightly pinkish stipe, and water-soaked grayish context; *R. handelii* has hyaline pleurocystidia and a stipitipellis with numerous caulocystidia; *R. jilinensis* has a yellowish green tinged pileus center, mild tasting context, and ocher to yellow spore print; *R. minutula* var. *minor* differs in the small (0.8–2.0 cm) pileus and pileipellis with primordial hyphae but without pileocystidia; *R. taliensis* is recognized by its pruinose opaque pileus, yellowish spore print, and spores with rare and isolated ornamentation; *R. pseudovesca* can be clearly perceived by its whitish spore print, numerous pleurocystidia >80 μm in length, and pileocystidia with 0.9–1.8 μm thick cell walls; *R. punicea* has a strongly acrid context that does not change color when old and injured; and

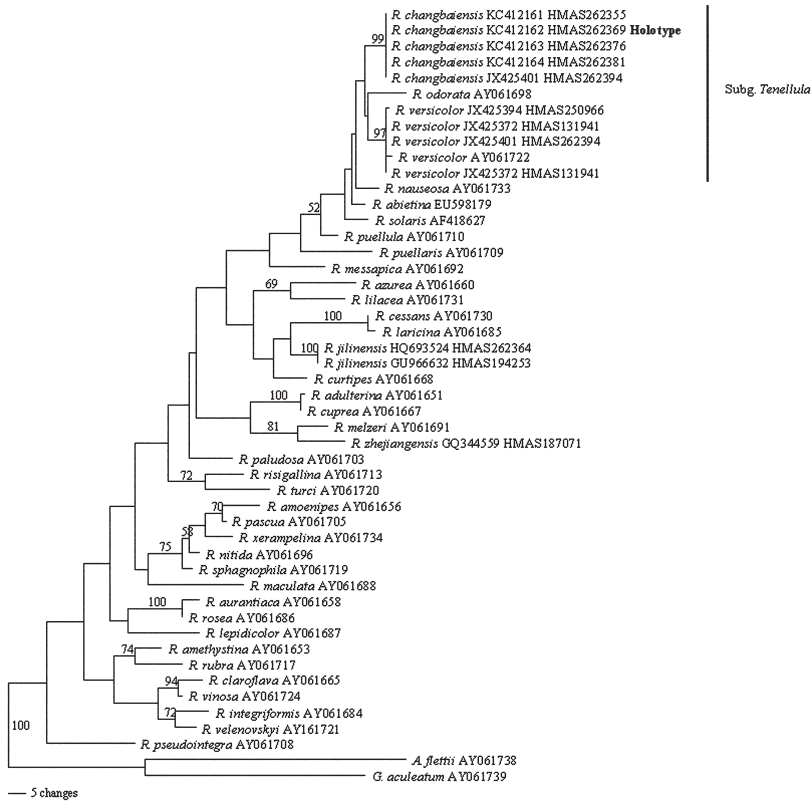


Fig. 3: Phylogenetic relationship tree of *Russula changbaiensis* and other related *Russula* species inferred from the dataset of ITS1-5.8S-ITS2 sequences. Consistency index (CI) = 0.486, retention index (RI) = 0.647, and tree length (TL) = 842. MP bootstrap support values >50% are indicated above the branches.

*R. zhejiangensis* has a bright red pileus with dark center, dark cream to ocher spore print, longer (≤74 μm) pleurocystidia, and a habitat of subtropical evergreen forest (Singer 1935, Chiu 1945, Bi & Li 1986, Ying 1989, Wang et al. 2009, Li et al. 2011, 2012).

**Phylogenetic results**

The dataset comprised 38 *Russula* taxa with particular attention paid to closely related *Russula* subg. *Tenellula* members and other red-capped *Russula* species. The sequence length was 534 nucleotides. In the MP analysis of ITS1-5.8S-ITS2 sequences, 264 characters were constant, 185 variable characters were parsimony-informative, and 85 characters were parsimony-uninformative. In the phylogenetic tree (Fig. 3), *R. changbaiensis* sequences formed a conspicuous clade, showing enough phylogenetic distance to support them as an independent taxon. *Russula changbaiensis* and most members of subg. *Tenellula* form a clade with a moderate bootstrap support of 52%, the same support as cited in Miller & Buyck (2002). The molecular analyses do support *R. changbaiensis* within subg. *Tenellula.* However, the phylogenetic relationships between *R. changbaiensis* and other subg. *Tenellula* taxa are still unclear; no stable topology can be established because there are so few corresponding sequences from dependably morphologically identified specimens.

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